mTOR-inhibitors may aggravate chronic hepatitis E

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Abbreviations: 4E-BP1, eIF4E-binding protein 1; eIF4E, eukaryotic initiation factor 4E; HEV, hepatitis E virus; IFN, interferon; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin.

Editorial

Although hepatitis E virus (HEV) is a much understudied pathogen, it is one of the most important causes of acute hepatitis worldwide. Based on calculations for genotypes 1 and 2, an annual incidence of 20 million HEV infections resulting in about 70.000 deaths has been estimated [1]. These two genotypes are endemic in developing countries and cause large-scale water-borne outbreaks [2], such as the very recent outbreak in Nepal (www.promedmail.org; archive number: 20140509.2461705). A hallmark for such outbreaks is the high morbidity and mortality observed in pregnant women, with fatality rates up to 25%. The underlying pathogenesis for this particular vulnerability of pregnant woman is only very poorly understood, [1-3] although progesterone receptor polymorphisms may play a role [4]. Genotypes 3 and 4 are, by contrast, zoonotic pathogens that are frequently detected in commercial pig herds, but also in wild boar and deer [2,3]. The consumption of un- or undercooked pork is, as consequence, a major risk factor for contracting hepatitis E. Accordingly, the south of France is considered to be a hyperendemic region because of the popularity of local delicacies, such as figatellu, that are prepared with raw pork [3,5].

In general, most HEV infections are asymptomatic and most symptomatic infections resolve spontaneously [2,3]. Nevertheless, some patients may evolve to fulminant hepatitis, explaining the reported overall mortality rates of 0.5-4% [3]. Since 2008, it is known that hepatitis E can evolve to chronicity in immunocompromised patients [6]. Chronic hepatitis E has since been observed in HIV patients and leukemia patients undergoing chemotherapy, but most cases are organ transplant recipients receiving immunosuppressive treatment [2]. About 30% of chronic infections in the latter group can be resolved by reducing the level of immunosuppression [7]. Commonly used immunosuppressive drugs in the transplant setting are corticosteroids, mycophenolate mofetil (MMP), calcineurin inhibitors (cyclosporin A and tacrolimus) and the mTOR (mammalian target of rapamycin) inhibitors such as rapamycin and everolimus. In this issue of the *Journal of Hepatology*, Zhou *et al*. demonstrate that the latter two drugs promote *in vitro* HEV replication through inhibition

of mTOR [8]. Thorough studies of the involved signaling pathways reveal that mTOR is part of an antiviral signaling pathway that inhibits HEV replication. This antiviral activity is mediated through the eIF4E-binding protein 1 (4E-BP1) directly downstream of mTOR.

In another recent study by the same authors, the *in vitro* effect of other immunosuppressive drugs on HEV replication was reported [9]. While steroids were shown to have no effect on viral replication, the calcineurin targeting drugs cyclosporin A and tacrolimus resulted in a pronounced proviral effect which was shown to be mediated by the inhibition of cyclophilins A and B. By contrast mycophenolic acid (the active component of mycophenolate mofetil, MMP) was shown to be an inhibitor of *in vitro* HEV replication [9,10]. This antiviral effect may be in line with a clinical observation that the use of MMP was associated with HEV clearance [11]. It should be noted though that this observation was based on a small number of patients.

These findings raise the question whether the immunosuppressive drug scheme should be adapted for patients with chronic hepatitis E. Should calcineurin and mTOR inhibitors be avoided and MMP (and possibly steroids) be preferred if a patient in need of immunosuppression has been shown to be HEV positive? Should such preferences be extended to non-infected patients who are at risk of contracting chronic hepatitis E (such as for example pig farmers)? One important caveat is that such recommendations would be solely based on *in vitro* findings that possibly do not take all aspects of hepatitis E pathogenesis into account. For instance, the *in vitro* anti-HEV activity of mycophenolic acid is mediated by an efficient depletion of intracellular GTP pools in cell cultures; an antiviral effect that can be easily reversed upon exogenously addition of guanosine [10]. It is however questionable whether such strong depletion of GTP pools by MMF is at all possible in the human liver [12]. Even if MMF would be able to deplete GTP pools in the liver to levels that may be sufficiently low to impact HEV replication, the virus may, in an immunocompromised environment, not necessarily be much limited in its replication. Mycophenolic acid inhibits also efficiently and completely the *in vitro* replication of a number of flaviviruses [13]. Yet in a murine model for flavivirus infection, we did not

observe any protective activity of MMF (our unpublished data). Similarly, addition of MMF to interferon for the treatment of interferon-non-responsive chronic hepatitis C patients proved ineffective in a clinical trial [14]. It will thus be important to explore the impact of these different immunosuppressive drugs on HEV replication in (a) relevant infection model(s) in animals. HEV replication was recently demonstrated in uPA/SCID mice of which the diseased liver had been repopulated with human hepatocytes [15]. This, and perhaps other, yet to be developed models, may be instrumental to demonstrate the differential (anti- and proviral) effects of the different immunosuppressive drugs. Retrospective studies on cohorts of chronic hepatitis E patients may allow to unveil whether a link exists between the clinical outcome and the choice of immunosuppressant(s). The low number of (reported) cases of chronic hepatitis E may complicate such exercise; yet given the recent increase in diagnosed cases, such studies may become feasible in the future.

One may put different hypotheses forward to explain the antiviral defense mechanism mediated by mTOR and downstream 4E-BP1. The protein 4E-BP1 is known to be a translational repressor, by interacting with the essential eukaryotic initiation factor 4E (eIF4E), mRNA translation is inhibited [16]. mTOR is known to phosphorylate and, thus to deactivate 4E-BP1, thereby releasing eIF4E which then initiates mRNA translation. More specifically, 4E-BP1 has important regulatory functions in the interferon (IFN) response [17]. Cells knocked-out for 4E-BP1 are remarkably resistant to viral infection because of a decreased threshold for IFN production [18]. This phenomenon is mediated by increased mRNA translation of IFN regulatory factor 7 (*Irf7*) which is normally suppressed by 4E-BP1. A similar mechanism may apply to the observed increase in HEV replication caused by rapamycin and everolimus. Indeed following inhibition of mTOR activity, 4E-BP1 may not be phosphorylated and thus remain associated with eIF4E. In this way, translation of *Irf7* or other factors would be inhibited, which may in turn result in a decreased IFN response and thus overall increased HEV replication. Other factors may of course be involved as well and alternative mechanisms may apply.

Most transplant patients with chronic hepatitis E that do not clear the virus by reducing immunosuppression are treated with an extended course of ribavirin [19]. Although this therapy is mostly effective, cases of treatment failure have been reported [20]. Moreover, long courses of ribavirin often result in side effects, including anemia. Modulation of the immunosuppressive drug scheme could be a very useful strategy to improve response rates to ribavirin, decrease the number of patients in need of ribavirin treatment and shorten the treatment time altogether. Today potent antiviral drugs are available for the treatment of infections with herpesviruses, the human immunodeficiency virus, the hepatitis B and C viruses and to a lesser extent influenza. Viral polymerase inhibitors (whether targeting DNA polymerases, reverse transcriptases or RNA dependent RNA polymerase) have been shown to be excellent targets for inhibition of viral replication [21]. Using a combination of highly potent and well tolerated antivirals, including nucleoside polymerase inhibitors, several studies recently reported a sustained virological response cure in >95 % of patients chronically infected with the hepatitis C virus [22]. This latter virus is, akin to HEV, a +ssRNA virus and encodes for several proteins (including a RNA dependent RNA polymerase) that may be good targets for pharmacological inhibition of viral replication [23]. In fact, it has been shown that some HCV nucleoside polymerase inhibitors (in particular the 2'*C* methyl series) inhibit the replication of yet other +ssRNA viruses including, but not limited to flaviviruses, enteroviruses and noroviruses [24]. It remains to be studied whether (some of the) HCV nucleoside polymerase inhibitors that have, or will reach the market, also inhibit HEV replication. In such case, they may be used (even off-label) either alone, or in combination with ribavirin, for the control of HEV infections. If such combination treatment would be sufficiently potent, there may no longer be a need to reduce immunosuppression to control chronic HEV infection in immunodeficient patients.

In conclusion, the work by Zhou and colleagues reported in the current issue highlights the potential importance of choosing the most appropriate immunosuppressant for use in patients with chronic hepatitis E. Confirmation of the observed *in vitro* effects in a suitable animal model for hepatitis E is awaited. Retrospective analyses (and if possible prospective studies) of immunosuppressive regimens

in chronic hepatitis E patients will also help to understand the potential effect of immunosuppressive drugs on HEV replication in the infected patient.

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